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GENETICS OF THE ENCEPHALITIS VECTOR, <u>CULEX</u> <u>TARSALIS</u>, FOR
POSSIBLE APPLICATION IN INTEGRATED CONTROL

Annual Report, 1979-80

Sister Monica Asman, Ph.D.

February 1980

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Culex tarsalis; reciprocal translocations; sterile-male; quality contol; mutants; pilot release studies; salivary gland chromosome mapping.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The projects here reported represent part of an overall program designed to change C. tarsalis genetically to inhibit its propagation in nature, and to render it less effective as a vector of disease.
A resume of progress for the year 1979-80 is as follows:
A. The number of maintained strains for genetic studies was increased;
B. Multiple-marker strains for genetic studies and identification of translocations increased to 17. An additional mutant was isolated,
C. Among the translocated strains that were given priorities for evaluation

as release material are 3 autosomal homozygotes and a "pseudohomozygote" constructed from 2 of these;

D. A field pilot-release study using sterilized males was carried out at one of our release sites outside of Bakersfield, CA.

E. Several experiments compared reproductive behavior of wild type and laboratory-reared samples of Cx. tarsalis;

F. Comparative studies evaluated longevity, mating behavior, and competitiveness of sterilized males in large outdoor cages;

G. Progress was made in the mapping of the salivary-gland chromosomes,

H. Mark-release-recapture studies were carried on throughout the spring and summer.

I. Quality control measures included: rearing of colines in an outdoor environment; monitoring of colonies by electrophoretic techniques; comparative studies involving different photoperiods and feeding patterns.

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Annual Progress Report 1979-80

Introduction

The primary goal of our research is the development of genetic mechanisms that could contribute to an integrated control system for Culex tarsalis. Abatement of this mosquito species is the principal approach to control Western Equine, St. Louis, and California encephalitis in California and the western United States. Since the vector is increasingly resistant to all currently known and accepted insecticides, we are pursuing autocidal control as an alternative method. More specifically, our primary efforts have been to genetically manipulate segments of this species so that self-destruction will follow when they are introduced into a native population. This destruction could be due to sterilization, to inherited semi-sterility or to the introduction of genetic factors that would either act as autocidal time-bombs or at least make the population less medically important.

Genetic control research is relevant because: 1) increasingly constrained laws have all but banned some of the established pesticides, and the use of others is unacceptable or severely restricted; 2) the development of new chemical agents is becoming more difficult each year due to cost and regulatory regimes; 3) "natural" pest control mechanisms often are disrupted, especially when predators and parasites of the target species are also killed by the use of chemicals; 4) the autocidal (genetic) approach offers a highly species-specific means of controlling a mosquito species; 5) an integrated approach to control Cx. tarsalis has yet to be perfected.

The 5 major categories of our research to date are described below:

A. Laboratory Colonies, Genetic Strains

1. Colonies maintained

Six wild type laboratory colonies of Cx. tarsalis are maintained for specific purposes (Table 1). These include 2 representative populations that originated from the Poso West field area in Kern County where our experimental stocks are released. The Poso West Colony was initiated in September of 1978 and is maintained in the laboratory. The second colony from that area is Poso West Quonset (PWQ). This population is maintained under natural environmental conditions in a large outdoor quonset-type cage in Kern County. As in nature the females that are outdoors went into a diapausing stage this winter. We are hoping that in early spring they will feed, and gravid females will oviposit to establish the next generation. In both populations, PWC and PWQ, we hope the genotypes are remaining close to that of the wild population. These lines will be used discreetly for outcrosses with our experimental material prior to release so that the genotypes introduced for competitive purposes will be close to the field population. The Berkeley colony is a composite of several California strains and also is maintained in an outdoor cage, where we hope overwintering females will give us eggs early in the Spring.

The Aedes colonies we maintain are used in a sterile-male project we are presently doing under a State grant. This work is carried out by a post-graduate assistant supported by that grant.

2. Mutant stocks

A total of 14 single-gene mutation stocks are also maintained (Table 2), including a new mutant, eagle, which will be described below. These mutations, which have been identified for their linkage relationships, are used for basic genetic experiments, as tracer elements in field work, and to construct multiple-marker lines (Table 3) which carry at least one genetic marker on each of the 3 chromosomes. We now have 17 multiple-marker stocks for possible use in genetically isolating chromosomal interchanges and other chromosomal aberrations.

The mutant, eagle, was first noted in males in the Chico laboratory colony in 1978; however, a pure breeding line was not established until recently. The mutant is thought to be spontaneous. It appears to be linked to carmine eye-color, and thus is on linkage group three. The gene(s) affects the antennae, giving them an eagle-spread phenotype. The wings are also affected in that they are arched towards the center of the abdomen rather than flat. Both sexes are affected and show good expression.

Since last year's report we also have determined that the mutant, spaced eyes (spe), is sex-linked. While we do have a pure breeding stock, the expression or penetrance does not occur in all sibs of a generation. However, the trait is well expressed in most progeny. It must still be determined if the mutant merely has poor penetrance or if the mutant is controlled by more than one gene. Once this is determined we will be able to construct an additional multiple-marker line with this mutant. In the past, 27 chromosomal interchanges were genetically recognized and 3 autosomal and 9 sex-linked translocations were established as lines with these multiple marker stocks.

Both mutations and marker stocks are essential to our work for various other reasons: they are necessary tools for understanding the basic genetics of the species, they serve to genetically identify the position of chromosomal breaks and re-attachments, they have application in the study of chromosome mapping of genetic factors, and can be used as tracer stocks for field experiments.

In the search for new mutants of Cx. tarsalis we have occasionally caged Cx. tarsalis with Cx. pipiens to see if fertile hybrids could be produced. Such hybrids when backcrossed to Cx. tarsalis could serve as bridges for introducing Cx. pipiens genes into a Cx. tarsalis colony for use in genetic studies. We succeeded in recovering fertile males from a Cx. tarsalis female X Cx. pipiens male cross, and successfully mated them to Cx. tarsalis females. We are attempting to repeat this study with mutant Cx. pipiens stocks.

3. Electrophoretic analysis of colonization

We have long realized that information necessary for a sound rearing program of colonies had to include: a) a detailed understanding of the life history of the organism we are rearing; b) adequate information on the genetic effects of culturing populations artificially; and c) knowledge of the effects of inbreeding and genetic variation among strains

on mating behavior with field mosquitoes. Thus as part of our 'Quality Control Program' an attempt is being made to identify genetic changes caused by selection processes that might occur in the process of colonizing wild populations, and that would impair the competitive effectiveness in future generations. We theorized that a systematic sampling of a wild-type population prior to exposure to laboratory conditions and again in each subsequent generation after rearing began in the laboratory, would possibly identify subtle genetic variants occurring on the enzyme level. Thus we initiated a program based on the screw-worm-mass rearing system to genetically monitor ecologically and behaviorally significant loci affected at the biochemical level. By using the 1-gel or 2-gel electrophoretic enzyme analysis technique we hope to study altering rearing conditions on gene frequencies. Such data could explain observed differences in the compatibility of field and laboratory strains. Specimens that have been sent to our collaborator, Dr. Houk, for analysis include 3 generations of laboratory reared Poso West collected from our release site, representatives of a Poso West colony established in 1978, and material from our Bakersfield Colony, a long-established colony from our Arbovirus Field Station. The results we have received to date are incomplete; however, the limited scan-graphs returned do illustrate differences in enzyme activity between wild and colonized Poso West material. While low isoelectric point enzymes (esterases 1-4) predominate in field-collected samples, colonized Poso West has a higher frequency of esterase 6 (Figure 1). Further tests and study will hopefully clarify this simple observation.

4. Quality control and rearing.

A constant effort is made to maintain the quality of laboratory stocks, and to improve their ability to compete successfully with wild mosquitoes.

a. Laboratory studies: Maintenance of colonies on a more natural light and temperature regime was attempted in environmental chambers. Both variables were changed biweekly and equated to the conditions at Bakersfield, although the maximum temperature was never allowed to exceed 86°F. A cube cage containing PWC-78, and a cube cage containing field collected Poso West mosquitoes were maintained in this manner. It did appear that adults kept in this manner lived longer than adults kept in the larger insectary; however, there was no difference in raft production and the generation time was slower on the variable regime.

No significant difference was observed between PWC-78 larvae reared on 'Bakersfield chow' (brewer's yeast, Tetramin, and alfalfa pellets) and rat chow, although there was less fouling due to organic matter in the pans in the former.

We also found no difference in the rate of blood feeding between mosquitoes presented a mouse at 9:00am and 3:00pm. The observation was made that mosquitoes fed better in the area kept under wet cotton than in a dry area. Perhaps a humidity gradient was established in this case.

b. Field studies: Logic would support the idea that mosquito colonies maintained outdoors in large cages should contain more characteristics of field mosquitoes. Such colonies could be established from laboratory stock, or from infusions of wild mosquitoes.

Cages were constructed at the Russell Tree Farm Reservation of the University of California near Berkeley for this purpose. Two screened cages 6'(W)X8'(H) and one screened cage 6'(W)X9'(L)X8'(H) are permanent strcutures at the site. Two 4'X4'X4' collapsible cages have also been constructed, and may be moved as necessary to different experimental habitats. Two of the quonset units at Bakersfield were also designated for outdoor colony maintenance. One quonset unit contains Poso West wild material maintained by frequent infusions of field collected material. The other unit contains the heterozygote translocation stock outcrossed to the PWC-colony. The Berkeley laboratory colony has been in the Russell Tree Farm outdoor cage since September, 1979. Refugia in the form of cans, boxes, tree branches and pipes have been placed in these outdoor cages to see if the population can overwinter under these conditions.

B. Genetic Studies

1. Autocidal systems

An autosomal translocation stock, T(2;3) 16A has been established following procedures of the alternative method for isolating translocation homozygotes that we developed and reported on last year. This system made use of 'marked' heterozygotes. We now have available 3 translocation homozygote stocks: T(2;3)5A, without genetic markers, T(2;3)15A, and T(2;3)16A. The latter 2 are marked with carmine and black-eye genes. We have crossed the 2 mutant marked strains and are investigating whether we can isolate from this hybrid stock a pure-breeding and reproductively vigorous strain from recombination between the 2 translocations crossed.

We have continued to maintain 3 sex-linked translocation hetero-zygote strains. With each strain we have made numerous attempts to recover translocation homozygotes. Inbreeding of T(1;2;3)1A, T(1;2)17A, T(1;2)19A, and other suspected heterozygotes have failed to yield viable translocation homozygote progeny.

A new method is being tested for isolating new translocation homo-zygotes captured through existing homozygotes. If this method can be successfully implemented, any strain of Cx. tarsalis can be irradiated and altered to include a translocation homozygote in a short time. The availability of such strains will place us closer to our goal of genetic replacement of undesirable traits with desirable ones in a field population. Figure 2 illustrates this proposed procedure which assumes that individuals heterozygous for both the old and the newly-induced similar translocations ("pseudohomozygotes") will be fully fertile like true translocation homozygotes.

We are attempting to determine if certain "pseudohomozygotes" could be fully fertile even if one of the translocations was not identical to the other. A cross between 2 rather different autosomal transloca-

tions was made for such purposes. The T(2;3)15A with recombination of 5.3+/- 1.1 between carmine and black eye was crossed with the T(2;3)16A with recombination of 1.6 +/- 0.4 between the same markers. The "pseudohomozygote" male progeny were mated with fertile laboratory stock females. Male fertility was determined by the hatchability of the rafts that resulted from these matings. The hatch rate of the 4 rafts collected from inbred wild laboratory stock exceeded 90%, and 13 of 20 rafts from "pseudohomozygote" matings exceeded 90%, demonstrating that "pseudohomozygotes" could be fully fertile. Thus, our capture procedure should be valid for a broad range of newly-induced translocations.

We have irradiated wild type laboratory strains in attempts to induce and capture autosomal translocations by our newly proposed method. We made crosses with irradiated wild type and either T(2;3)15A or T(2;3)16A homozygote strains. We have examined 309 egg rafts parented by potential F-1 "pseudohomozygotes". Among these, 11 high hatch rafts were found. Completed tests on 7 of these have given no evidence of a newly-induced autosomal translocation. Work in this area is continuing with a larger scale screening program planned for the near future.

The mating competitiveness of T(2;3)15A homozygote, T(2;3)16A homozygote and T(2;3)15A/T(2;3)16A hybrid males (all mutant phenotypes) is being evaluated in laboratory cages. In these trials wild phenotype males competed with mutant males in 1 cage and with mutant translocated males in a second cage. The results of the first cage would indicate the effect of the mutant alone while those from the second cage would include effects of the mutant plus the translocation. The results to date are entered in Table 4.

As the data show, the mutant males out-competed the wild males for mutant females. However, the mutant translocated males, T(2;3)15A and T(2;3)16A were less competitive than wild. The wild males of genotypes heterozygous for the mutants were more competitive than either the mutant or mutant translocation hybrid, T(2;3)15A/T(2;3)16A males. The translocation hybrid males, however, did as well as the mutant males and would be expected to be more competitive than wild males. The data suggest that these hybrid males, T(2;3)15A/T(2;3)16A, would be the best choice for competition studies in larger indoor and outdoor cage studies this summer.

A series of outdoor cage trials with translocation strains for 1979 were designed and scheduled for 1979, but numerous problems in maintaining pure breeding and reproductively vigorous homozygote lines forced cancellation. We now have divided the lines of our translocation homozygote stocks and hope thereby to control contamination. The "reproductive vigor" problem will hopefully be solved by mixing the homozygotes for a pseudohomozygote as described.

2. Genetic replacement system

In our endeavor to develop a genetic replacement system for Culex tarsalis the mutant markers, carmine eye and black eye, are associated with 2 homozygote translocations to facilitate isolation and laboratory maintenance. Males of these 2 translocation stocks were recently tested for competitiveness in laboratory cages.

Competition crosses were established in 17 cm wide X 17 cm high cylindrical cages in pairs. The first cage contained mutant females with wild males in competition with mutant males. The companion cages contained mutant females and wild males in competition with mutant translocated males. The mosquitoes were two-day-old virgins when the cages were set up. When they were four-days-old the females were bloodfed. On the day following bloodfeeding females were separated from the males and placed into smaller cages singly for oviposition into shell vials. As the translocation systems tested were associated with normal hatch, the hatchability of rafts would not indicate the type of mating. The rafts were hatched and the progenies raised. The type of mating that had occurred was determined by progeny examination since the two types of males in each of the competition cages would give distinctly different progeny phenotypes.

In the first pair of crosses the T(2;3)15A translocation was tested (Figure 3). In one cage 44 mutant females were caged with 44 wild males and 44 mutant males. Progeny examination indicated that 14 of 24 rafts resulted from matings with mutant males. In the companion cage where mutant and mutant-translocation males were competing for mutant females, only 7 of 21 rafts were attributed to matings involving mutant-translocation males. These results show that the translocation puts the mosquitoes at a competitive disadvantage.

In the second pair of crosses the T(2;3)16A translocation was tested in similar fashion (Figure 4). In the first test where mutant females were caged with wild and mutant males, the mutant males showed a competitive advantage with 15 of 20 matings attributed to them. In the companion cage holding wild and mutant-translocated males, only 3 of 23 matings involved the mutant translocated males. Quite surprisingly all three of the progeny groups gave evidence that multiple insemination had occurred. Apparently the T(2;3)16A translocation system is associated with a deficiency in monogamy as well as a competitive disadvantage.

In the third pair of crosses a hybrid translocation system of both T(2;3)15A and T(2;3)16A was tested (Figure 5). The first cage contained mutant females and wild and mutant males. In this case the wild males were heterozygotes, carrying both recessive mutant genes but of wild appearance. Only 3 of 14 matings were attributed to mutant males, indicating a distinct heterozygote advantage of the wild males. In the companion cage the wild heterozygote males outcompeted the mutant-translocation males, with 14 of 20 matings. Nevertheless the mutant-translocation males were more competitive against wild than the mutant males in the companion cage. In earlier competition tests these hybrid translocation homozygotes were found to be superior to the regular wild males. Based on those data, these hybrid males should be considered for possible use in replacement schemes and as candidates for testing in larger laboratory and outdoor cages this coming season.

3. Chromosomal mapping

Progress in the isolation, identification, and mapping of the large salivary polytene chromosomes of Cx. tarsalis will contribute much to our program in basic genetics. The 3 chromosomes have been

identified as distinct and separate entities and specific banding patterns can now be recognized and described.

Within the next year we expect to publish the first paper on the salivary chromosomes of this species. Hopefully, the modified techniques that we have established will also help solve the problems encountered in the preparation and spreading of polytene chromosomes in other Culex and Aedes species where past efforts have been fruitless.

C. Sterile Male Program

For the past 2 years we have studied the feasibility of using the sterile-male technique as a support system to the insertion of translocated stocks to control Cx. tarsalis. The procedures followed were suggested by the International Atomic Energy Agency (IAEA), which has co-ordinated several international programs to control insect pests. We have completed much of Phase I and parts of Phase II of the plan (Figure 6), and are continuing research in these areas in the coming year.

As reported last year extensive sterility curves were established by irradiating young (0-24 h) adult males with a CO-60 source which gave a dose rate of approximately 160 r/min. Mating competitive tests in laboratory cages consistently indicated that 5 krads from this source produced highly sterile and competitive males. We found a mean of 43% egg hatch resulted when irradiated and non-irradiated males competed in a 1:1 ratio, as compared to a 92% hatch in the control unirradiated cage, and 3.0% in the control irradiated-male cage. Large outdoor-cage tests with males at a 1:1 ratio resulted in 50% low-hatch rafts, an average of 3.3% hatch/raft. Other tests demonstrated that there was a significant increase in sterile rafts when the frequency of the sterile males was increased; however, changing the sex ratio did not significantly alter the mating competitiveness of sterile males on the whole.

This past year several laboratory and field-cage experiments, as well as a pilot field trial, were completed using the sterile-male method.

1. Tests for multiple insemination of females by irradiated males

The objective of this study was to determine if multiple insemination would occur if females were exposed simultaneously to irradiated and unirradiated males, or if females were first exposed to irradiated males and 3 days later to unirradiated males. The irradiated males were genetically unmarked Knights Landing stock, and the unirradiated males carried a black-eye (ble) marker, a recessive trait to normal eye color. All females used in the experiment stemmed from the black-eye colony. Thus black-eyes in the progeny indicated fertilization by the unirradiated-mutant males, and normal eye in the progeny indicated fertilization by irradiated normal-eyed males.

In 2 of the 38 cases, Cx. tarsalis black-eyed females simultaneously exposed to irradiated KL males and unirradiated black-eyed males oviposited egg rafts containing both normal-eyed and black-eyed

individuals. Both examples were from low-hatch egg rafts, and none occurred among the high-hatch egg rafts. No mixed-hatch rafts occurred when females were exposed to a group of unirradiated black-eyed males after 3 days of exposure to a group of irradiated KL males. The mixed-hatch egg rafts in the study could well have resulted by a female first mating with an irradiated male and then with an unir-radiated male within a limited time period. Multiple insemination involving males of the same strain would be undetectable and could have occurred without our knowledge. It is questionable if the application of such results to a field situation is valid, as the chances are small that a wild female will encounter an irradiated male whose sperm supply is depleted and then in a short time encounter a normal male.

2. Longevity of irradiated vs. unirradiated males

It is important in the sterile male approach to control Culex tarsalis to determine the longevity of irradiated males (and females exposed to those males) and unirradiated males (and females exposed to those males).

We placed 20 males irradiated at either 5.0kR or 7.0kR when less than 24 hours old, or 20 unirradiated males, with 20 virgin females of the same age and strain in 1-gallon cylindrical cages. Each cage was provided with water and 2 sugar cubes. The 3 replications of each treatment and strain (Berkeley and PWC-78) were randomized on a shelf in the insectary. Significant differences in longevity could be attributed to irradiation dose, strain and cage, but not to sex (Table 5).

Mosquitoes irradiated at 5.0kR lived longer in each case than either mosquitoes irradiated at 7.0kR or unirradiated mosquitoes (Table 6). Berkeley strain mosquitoes lived longer than PWC-78 strain mosquitoes. The difference among cages was the most important source of variation, and probably reflected differences in suitable microhab-itats even on one shelf in the insectary. This demonstrates the necessity of replicative experiments. When the 3 replications were lumped, cage effects were eliminated and the differences were significant.

In conclusion, the data suggest that Cx. tarsalis irradiated at 5.0kR live longer than unirradiated Cx. tarsalis; however, it is likely the results were biased by individual cage distinctions. In the future, more replications will be used, and cages will routinely be rotated on the shelves.

3. Determining mating ability of irradiated males

The objectives of this study were to test if polygyny (a male consorting with more than one female) occurred with equal frequency among irradiated and unirradiated Cx. tarsalis, and to determine if an unembryonated egg raft could only have been oviposited by a female that was mated by an irradiated male. Two laboratory colonies were used in the study: KL, and PWC-78, a strain colonized for 1 year from Poso West. The comparison of polygyny was tested by placing 1 two-day-old irradiated male and 1 two-day-old unirradiated male with

10 virgin females of the same age and strain in 2 separate cages. Females were removed after 10 days and their spermathecae were checked for sperm.

The incidence of egg rafts with no embryonation was tested by introducing equal numbers of irradiated KL males and virgin KL females into cube cages 30 cm on a side. In each of two cages, 50 2-day-old males irradiated at 5.0kR were placed with 50 2-day-old virgin females. A third cage contained 50 2-day-old males irradiated at 6.0kR, and 50 2-day-old virgin females. A control cage contained 40 2-day-old unirradiated males and 40 2-day-old virgin females. All adults were separated by sex within 24h of emergence. Males were exposed to gamma radiation produced by a Mevatron[R] linear accelerator at a rate of 250R/min. After feeding on a chick that was provided as a blood source in each cube cage, female mosquitoes were removed and placed individually in 10 dram vials for oviposition. Resulting egg rafts were held for 3 days, then scored for hatch rate and embryonation rate.

To determine the source of unembryonated egg rafts, KL males less than 24h old were irradiated at 5.5kR from a cobalt 60 source at 160R/min, and placed in a cylindrical cage 36.0cm high and 16.5cm in diameter with 200 virgin KL females of the same age. Females which fed upon a mouse provided as a blood source were isolated individually in 10 dram vials for oviposition. Resulting egg rafts were held for 3 days, then scored for hatch rate and embryonation rate. After each female oviposited, her spermathecae were removed and checked for the presence of sperm.

About 25% more females were mated and about 30% more females were fully inseminated by unirradiated males than by irradiated males (Table 7). An analysis of variance comparing the 50 cages containing unirradiated males with the 50 cages containing irradiated males indicated significant differences in both the number of females mated by 1 male ($t = 2.567$; $P < 0.01$; $DF = 98$), and the number of females fully inseminated by 1 male ($t = 3.056$; $P < 0.005$; $df = 98$).

A KL male mated with more KL females than a PWC-78 male with PWC-78 females, however there was little difference between strains in the number of females fully inseminated (Table 8). An analysis of variance comparing the 50 cages containing KL males and females with the 50 cages containing PWC-78 males and females showed a significant difference in the number of females mated per male ($t = 2.679$; $P < 0.005$; $df = 98$), but no significant difference in the number of females fully inseminated per male ($t = 0.305$; $P > 0.35$; $df = 98$). Unembryonated egg rafts occurred more frequently among females placed in cages with irradiated males than among those caged with unirradiated males (Table 9). Hatch rates were different when calculated to include or exclude such rafts. In tests determining whether unembryonated rafts came from virgin or mated females, 28% of 135 egg rafts were unembryonated,which is comparable to the sterility rate found in matings with males irradiated by the linear accelerator. All of the embryonated rafts and 50% of the unembryonated rafts were laid by females that contained sperm in their spermathecae. An analysis of variance gave no significant difference in mean size (Table 10) of

egg rafts from inseminated and uninseminated females (t = 0.879;
P > 0.15; df = 133).

In summary, an irradiated male Cx. tarsalis was capable of mating
and fully inseminating more than 1 female in a small laboratory cage,
although significantly fewer than could be mated and inseminated by
an unirradiated male. In earlier tests (1978-79 Report) we observed
that irradiated males competed equally against unirradiated males
for virgin females in the initial mating period, but not later. Those
results could be explained by the difference in polygyny of irradiated
versus unirradiated males. PWC-78 males were less likely to mate
with caged females than were KL males, but were almost equally capable
of fully inseminating caged females. The difference in relative
mating success could be attributed to the more recent colonization
of the PWC-78. More unembryonated egg rafts were produced by females
caged with irradiated males than by those caged with unirradiated
males. The reason for this is not understood. It is possible that
females which laid unembryonated egg rafts and that were observed to
be inseminated could have copulated with a depleted male, thus
receiving little or no sperm but being monogamized. We have shown
that inseminated females can produce unembryonated egg rafts. Includ-
ing the unembryonated egg rafts produced by inseminated females
lowered the mean hatch rate by 0.4% in estimates of fertility, and
the mean embryonation rate by 1.0% while increasing the sample size
from 97 to 116 egg rafts.

4. Mating competitiveness of irradiated male Cx. tarsalis in quonset studies.

The purpose of these studies was to evaluate the competitive
ability of irradiated males in large outdoor field cages at various
release ratios.

Release 1 had either a 1:1 ratio or a 2:1 ratio of males irradiated
at 5.0kR to unirradiated males. In one cage, 800 irradiated males
and 800 unirradiated males were released with 800 virgin females.
In a second cage, 1600 irradiated males and 800 unirradiated males
were released with 800 virgin females. A third cage contained 800
irradiated males and 800 virgin females.

Release 2 had a 9:1 ratio of males irradiated at 5.5kR to unirrad-
iated males. In cages 1 and 2, 1800 irradiated males and 200 unirrad-
iated males were released with 1000 virgin females. Cage 3 contained
700 irradiated males and 700 virgin females. Cage 4 contained 1000
unirradiated males and 1000 normal females.

Release 3 had a 9:1 ratio of males irradiated at 6.0kR to unirrad-
iated males. In one cage, 1800 irradiated males and 200 unirradiated
males were released with 1000 virgin females. A second cage contained
1500 irradiated males and 1500 virgin females.

Mating competitiveness was estimated by the ratio of the number
of egg rafts sired by irradiated males (those with less than 50%
hatch = low hatch) to that sired by unirradiated (those with greater
than 50% hatch = high hatch) after compensating for the release ratio.

The observed hatch and mating competitiveness of the irradiated

males in each experiment are shown in Table 11.

Results: The differences in mean hatch rates in the 3 cages in release 1 were significant. The number of low hatch rafts observed in the competition cages did not differ significantly from the expected ratios ($\chi^2_{1:1:1}$ = 0.556; df = 1; P = 0.46 and $\chi^2_{2:1:1}$ = 0.010; df = 1; P = 0.92). The mean raft sizes were 114 for the 2:1:1 release, 123 for the 1:1:1 release, and 121 for the 1:0:1 release. These differences were not significant. There was no correlation between hatch rate and raft size (r = 0.025). There was a significant correlation between hatch rate and embryonation rate for the 2:1:1 release (r = 0.97), the 1:1:1 release (r = 0.99), and the 1:0:1 release (r = 0.84).

The mating competitiveness of the irradiated males in release 2 was only 0.37. This might have been the result of the high hatch rate in the sterile control cage where 8 of the 55 rafts collected had hatch rates of 89-100%. The high hatch rafts could have been the result of incomplete irradiation of the males, or a source of undetermined contamination.

The estimated mating competitiveness of the irradiated males in release 3 was 1.33. The observed data did not differ significantly (χ^2 = 0.046; df = 1; P = 0.83) from the expected ratio. The sterile control cage appeared to be contaminated as 5 of the 34 rafts collected had hatch rates of 88-100%, or again there was incomplete irradiation of the males. As the only two egg rafts collected on the first day of sampling had 100% hatch and all mosquitoes released were of equal age, it is possible that some inseminated females from a previous experiment could have remained in the cage at the time of this release.

5. Sterile-male Field Release

a. Pre-release experiments

The Breckenridge study site is located in the Sierra foothills about 13 km east of Bakersfield. It consists of 3 canyons which are separated by ridges approximately 60 m high. Waste water from nearby oil is disposed of by being pumped into a series of shallow ponds in each canyon and allowed to evaporate, or is sprayed on the hillside to promote growth of pasture grass.

The area supported a small breeding population of Cx. tarsalis and was relatively free of other mosquito species. Breeding was most abundant in the outflow areas, under brush which periodically blew into the ponds, and in waterfilled hoofprints left by horses and cattle which grazed at the site. The breeding areas were temporal, and often changed rapidly when the source water was shut off.

Cx. tarsalis abundance was measured by operating CO_2/light traps at trap sites in all 3 canyons (Figure 7). Canyon B had the highest female populations initially. The peak seasonal abundance of females in Canyon C was during the latter half of July. Canyon A typically

supported the lowest female population of the 3 canyons. The female population population in all 3 canyons dropped dramatically after mid-July. The decrease coincided with the elimination of water out-flow from the lower pond of canyon A and from sprinkler heads at the base of canyon B.

A mark-release-recapture experiment was conducted 1 month prior to the release of irradiated males to determine the extent and direction of dispersal between the 3 canyons at Breckenridge (Figure 8). Adult Cx. tarsalis collected in CO_2/light traps in each canyon were marked with fluorescent dusts and released into the canyon from which they were captured. Recovery collections were made on the 2 nights following the release at the same trap sites. From the females recaptured it can be assumed that 1 of 3 females from canyon A migrated to canyon B, 1 of 9 females from canyon B migrated to canyon C, and 1 of 2 females from canyon C migrated to canyon B. Intercanyon migration by males could not be studied, as no males were recaptured.

Vapona was applied aerially at a rate of 3 gallons per acre to canyon B at 6:30 AM the day prior to the initial release of irradiated males. The purpose of the fogging was to decrease the population of inseminated females in the release area, and to lower the existing male population. It was anticipated that such a procedure would have a minimal effect on the indigenous larval and pupal population. To monitor the treatment, adult Cx. tarsalis males and females reared from pupae collected at Poso West, another study area near Bakersfield, were sequestered in 1 gallon cartons that were screened at both ends. Three cartons representing 3 age groups were placed horizontally in the open on the light standards, in tamarisk trees, and at the base of cat-tails in the treated canyon B and in the untreated canyon C.

No more than 42% mortality was observed among mosquitoes held in any carton in the treated canyon, although mortality was significantly higher for all categories of mosquitoes from the treated canyon except those mosquitoes in cartons placed in the tamarisk trees (Table 12).

b. Release of sterile males

All mosquitoes used in the field release were reared from wild pupae collected at Poso West. Each afternoon, adult males less than 24 hours old were exposed to 6 kiloroentgens of gamma radiation from the Metavatron linear accelerator at Bakersfield Community Hospital at a rate of 250 roentgens per minute.

A total of 13,500 irradiated males were released into canyon B before 7 AM on August 2nd, and August 3rd, 1979. [The irradiated males were released in the area of light traps 2, 4, and 5.] One third of all males released were marked with fluorescent dusts (Figure 9).

Ten percent of all irradiated males from both days were released into a large outdoor cage with an equal number of virgin females of equal age as a sterile control. Twenty-nine of 34 egg rafts gathered from the cage after the release had hatch rates of less than 20%. To evaluate the compatibility of mating between Breckenridge females and unirradiated Poso West males, 60 3-day-old virgin females reared

from pupae collected at Breckenridge were placed into a 60 cm cube cage with 120 3-day-old Poso West males. A control cage contained 60 virgin females and 120 3-day-old males reared from pupae collected at Poso West. After 5 days, the spermathecae were removed from 20 females from each cage. The insemination rates of the 2 groups of females by unirradiated Poso West males were not significantly different.

c. Post-release Monitoring

CO_2/light traps in the 3 canyons were operated nightly for 6 nights following the initial release (Figure 10). Live females which did not contain undigested blood in the gut were removed from the samples each day and placed into 30 cm cube cages. A restrained chick was provided as a blood source in each cage. Females which took a blood meal were transferred to individual 10 dram vials for oviposition. Each raft was examined after 3 days for hatch rate and embryonation rate.

The females collected in CO_2/light traps at Breckenridge yielded 202 embryonated egg rafts. There was no difference between canyon B and canyons A and C in hatch rate or embryonation rate for egg rafts laid after the release. This might be attributed to the movement of females inseminated by irradiated males in canyon B.

Females captured after the release of irradiated males produced more low hatch and medium hatch egg rafts than those females captured prior to the release (Table 13). Prior to the release, only 1 of 50 embryonated egg rafts had low or medium hatch. After the release, 34 of 202 embryonated egg rafts had low or medium hatch. Our attempt at adulticiding was unsuccessful, so many of the high hatch egg rafts could have been the result of females present prior to the release. We also know there was incomplete sterilization of released males, as the percent egg hatch in the control sterile cage was much higher than the 97% sterility we anticipated.

Six marked males and 6 unmarked males were recaptured from traps in canyon B. As the number of unmarked males did not exceed the number of marked males by a factor of 3, the release ratio could not be determined. No marked males were found in traps in canyons A or C indicating no male movement between canyons. The population remained low after the release but the effects of sterile males could not be measured.

From this initial field release we have learned that the application of this technique is limited by our inability to rear and sex large numbers of competitive mosquitoes for release, by the unavailability of a suitable irradiation source near the release site and field station, and by the need for additional baseline information on the ecology and population dynamics of individual field populations. Continued efforts in these areas hopefully will improve future release trials. In addition, this release covered only over 1 generation; multiple releases through several generations could well increase the percentage of sterile egg rafts recovered from a native population.

6. Genetic sexing system

In order to expedite rearing procedures where only males are needed for release we continued to attempt to construct a genetic sexing system for Cx. tarsalis. Such a system would eliminate the need for time and labor consuming mechanical separation. In addition, genetic sexing would allow release of pre-adult stages which would give an accommodation period for the released mosquitoes before they compete for mates in the field. If genetic sexing can be achieved during early larval stages, space and time requirements for rearing would be much reduced.

The successful development of a genetic sexing system with dieldrin resistance has been reported for Anopheles gambiae (Curtis et al., 1976, Mosquito News 36: 492-498). We are attempting to isolate dieldrin resistant and susceptible strains. Once isolated, the dieldrin resistant gene will be mapped and linked to the male-determining gene via a translocation. The system would have dieldrin resistant males and dieldrin susceptible females. Insecticide application to the early-stage larvae would eliminate the females and leave the desired males for rearing and releases.

We have surveyed several established colonies for dieldrin suscep-tibility (Table 14). None of the strains exhibited a great deal of resistance, but the 3 mutant stocks, fringe, bronze and carmine-charcoal, showed more resistance than others. A program is being continued to select resistance from single families primarily from field-collected samples where resistance to this insecticide was observed.

D. Biological and Ecological Studies

The single most important contributing factor to lack of success in any genetic release program for insect control is a lack of infor-mation on the basic biology and ecology of the species in question. For this reason we have continued to carry out studies on the mating behavior of Cx. tarsalis in addition to those reported above on com-petition between irradiated and non-irradiated males. Our associates at the Bakersfield Field Station continue to carry out ecological studies at our field release sites outside Bakersfield, Poso West and Breckenridge,by means of mark-release-recapture studies. These are reported below.

1. Mating trials between adults of different geographic populations

Groups of 60 Breckenridge and 60 Poso West females were each placed in separate small cages with 120 Poso West males. The insemination rates were 40% for Breckenridge females, and 30% for Poso West females. Statistically, the difference was not signifi-cant. A second test did a reciprocal evaluation of matings where females from McVan and Poso West were mated to males from the same areas. The insemination rate of McVan females with Poso West males was 72%, and that of Poso West females with McVan males was 54%. The test was done in a large outdoor quonset cage. The difference in the insemination rate was significant, and in a very preliminary way indicated mating behavior could be a factor to be considered in working

with various isolated populations of Cx. tarsalis. One interesting observation related to the second test was that the McVan females in this test were larger that the Poso West females.

A field mark-release-recapture study was designed in order to determine if females reared from a laboratory colony or hatched from field-collected pupae would preferentially mate with males of their own kind, and to make further observations on the dispersal and survival of laboratory versus wild adults. Laboratory mosquitoes were from the KL/T-78 colony, and the reared field samples were collected at Poso West as pupae. Adults of similar age from both groups were marked with various dyes and released at 3 points. Recovery collections were made for 10 days, using 18 CO_2/light traps. Recovery collections totaled 25,070 females and 12,369 males, including 148 marked females and 8 marked males. Recapture rates were lower than expected, possibly due to extremely high temperatures. Survival rates were "normal" for that time of the year (June); mean dispersal rates were 127 m for laboratory females, and 270 m for reared wild females. Of the 28 egg rafts recovered from marked females, no rafts appeared to have been sired by laboratory-reared males giving support to the possibility that the female does discriminate in mating. It is also possible that the males from the KL/T-78 were not carrying the translocation or were not competitive with wild males.

2. Studies to determine the effect of fluorescent dusts

Marking with fluorescent dusts has been one of the tools employed to estimate population densities, migration, survival, and behavior in the field. Tests were done to evaluate the effects of "dust" application on adult mosquitoes. One experiment compared the effects of Helecon and Radiant fluorescent dusts by releasing marked and unmarked adults from the 'charcoal' mutant colony. Three groups of 'charcoal' adults were released at a specific site; 1 group was marked with red Radiant dust, 1 with yellow Helecon dust, and the last was left unmarked. Recovery collections covered 5 nights following release. Recaptures totaled 16.3% of released females and 1.3% of released males. There were no significant differences between the 3 groups. Mean dispersal distance for females of all 3 groups was 102 m.

A second test compared the effect of fluorescent dusts by releasing both marked and unmarked adults from the 'charcoal' mutant colony. Survival and dispersal of laboratory (charcoal) versus field (reared) adults of similar age were measured. All mosquitoes were released at 1 site, and recovery collections covered 5 days. A total of 10,391 female and 5,991 male adults were collected, a far greater proportion of males than in previous studies. Mean distances of dispersal did not differ significantly, although "field studies" consistently moved more than laboratory adults. Females of both groups moved more than males. The direction of dispersal different significantly between the 2 groups as laboratory adults tended to move downstream and field adults tended to move upstream from the release point. No differences in survival or dispersal were detected between the dusted and non-dusted charcoal adults.

The above experiment was repeated 4 months later (September). Approximately 10,000 adults were released and recaptures were made for 7 days. There were no significant differences in recaptures or dispersal of marked versus unmarked 'charcoal' adults. In fact, the number of each group recaptured never differed by more than 2 for either sex on any given day. The results confirmed the observations made in the first trial (May). As observed in the earlier study, laboratory females tended to disperse less than wild ones, although the differences were not significant in this study. The mean dispersal distances were greater and again reflected the preference of all adults for the upstream area. Release and collection data are summarized in Tables 15 and 16.

A test was made in the large outdoor quonset cages to determine: if dusted males survived as well as non-dusted ones over a 72 hr period, and if dusts affected the insemination rates of females. There was no significant difference in the number of undusted and dusted males recovered at 3 specific time intervals up to 72 hr. There also was no significant difference in the insemination rates (57% and 52%) between females mated by dusted males and non-dusted males.

3. Determining field survival and dispersal

A total of 23,340 Cx. tarsalis adults dusted with 9 different fluorescent dusts were released to compare the survival and dispersal of different age cohorts and to estimate survival, population size, and emergence in the month of July. Because of the small number of re-captures in individual cohorts, it was not possible to compare survival of the different age groups. In fact, in order to compute any survival rate, it was necessary to combine data from all cohorts. This was legitimate as the method assumed constant daily mortality, regardless of age. It was estimated that there was a mean daily survival rate of 61%, or a mean daily mortality loss rate of 39%. The population fluctuated widely during the 10-day recapture period: female collections ranged from 3,425 to 20,208 per day, and male collections went from 472 to 11,960. Estimates of daily emergence were 341,086 and 332,633 respectively. Differences in mean dispersal distance were not significant. The July mark-release-recapture data are seen in Table 17.

E. Updated Biographical Sketch and Bibliography

Principal Investigator and Genetic Staff

1. Presented papers (1979)

Asman, S.M. "Status of Research on Genetics of Culex tarsalis directed towards Integrated Control". CMCVA.

McDonald, P.T. "Effects of Laboratory Colonization on the Reproductive Abilities of a Field Collected Culex tarsalis Population". CMCVA.

Ainsley, R. and M. Asman. "The Relationship of Sex Ratio and Mating Competitiveness of CO-60 Irradiated Culex tarsalis Males". CMCVA.

Zalom, Frank and S.M. Asman. "Mating Competitiveness of Irradiated Males of <u>Culex</u> <u>tarsalis</u> in a California Field Test". ESA Meeting.

2. Publications (1979)

Asman, S.M. 1979. Status of research on the genetics of <u>Culex</u> <u>tarsalis</u> directed towards integrated control. Proc. Calif. Mosq. and Vect. Cont. Ass'n. 47: 56-59.

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Ainsley, R.W., S.M. Asman, and R.P. Meyers. 1979. The optimal radiation dose for competitive males of <u>Culex</u> <u>tarsalis</u>. J. Med. Ent. (In Press).

Ainsley, R.W. and S.M. Asman. Genetic fitness of the mutant, carmine eye, in <u>Culex</u> <u>tarsalis</u> in the laboratory. Mosquito News (Accepted for publication)

Asman, S. Monica and H.A. Terwedow. Initial studies on the genetics of <u>Aedes</u> <u>sierrensis</u>. Mosquito News (Accepted for publication)

3. Publications in Preparation

F.G. Zalom, R.P. Meyer, S.M. Asman, M.M. Milby, and W.C. Reeves. Release of irradiated male <u>Culex</u> <u>tarsalis</u> into a natural population. (To be submitted to J. of Econ. Entomol.)

F.G. Zalom, S.M. Asman, and R.P. Meyer. Determining mating ability
of irradiated male Culex tarsalis (To be submitted to Ann. of
Ent. Soc. Amer.)

F.G. Zalom, S.M. Asman, and R.P. Meyer. Mating competitiveness
of irradiated males of Culex tarsalis in quonset studies.
(To be submitted to Mosq. News)

F.G. Zalom, S.M. Asman, and J.E. Fields. Tests for multiple
insemination of females by irradiated males in Culex tarsalis
(To be submitted to Mosq. News)

F. Personnel Receiving Contract Support

Dr. Paul McDonald (Assistant Research Entomologist III) (100%)

Dr. McDonald has been on the program from its conception in 1974.
Prior to that time he had three years of field experience with
Aedes aegypti control in Africa.

Jane Fields, Laboratory Assistant I (50%)

G. Personnel Involved Supported by Other Sources

1. Sister Monica Asman, Principal Investigator (80%)
2. William C. Reeves, Epidemiologist (10%)
3. James L. Hardy, Virologist (10%)
4. Edward J. Houk, Insect Physiologist (10%)
5. Marilyn Milby, Statistician (10%)
6. Frank G. Zalom, Post-graduate Research Entomologist (100%)
7. Jane Fields, Laboratory Assistant (50%)
8. Naomi Rosenthal, Laboratory Assistant I (50%)

H. Collaboration Involved in Project

This overall research project requires a multidisciplinary approach,
and one of the greatest assets for a successful conclusion of our
work lies in the collaborative efforts among the participating
personnel. Collaborators include members of the staff from the
Department of Environmental and Biomedical Health Sciences,
Berkeley, and the personnel at the Arbovirus Field Station at
Bakersfield. We believe that the multidisciplinary team that
contributes to both laboratory and field investigations gives us
a unique ability and a rational approach to the investigations
relative to the specific objectives we have.

Table 1. Wild-type colonies maintained in the laboratory

Culex tarsalis	Geographic source
Knight's Landing	Yolo County
Chico	Butte County
Poso West Wild (PWQ)	Kern County
Poso West Colony (PWC)	Kern County
O.P. Susceptible	Fresno County
O.P. Resistant	Fresno County
Berkeley	Composite of several California strains

Other colonies

Culex pipiens	Sacramento County
Aedes sierrensis	Solano County
A. sierrensis	Fresno County
A. sierrensis	Contra Costa County

Table 2. Monofactorial mutants of _Culex tarsalis_ that are
maintained as laboratory colonies.

Colony designation	Abbreviation
black eye	ble
carmine eye	car
mulberry	mul
microcephalic	mic
bleached ocelli	bloc
fringe wing	fr
wide wing	ww
charcoal	char
ebony	eb
eagle	eag
melonotic-1	mel-1
melonotic-2	mel-2
bronze	brz
spaced eyes	spe

Table 3. Multiple-marker lines of Culex tarsalis that are
maintained for genetic studies.

Linkage group I	Linkage group II	Linkage group III
sex (gene determined) (M)	black eye (ble)	carmine (car)
fringe (fr)	ble	car
mulberry (mul)	ble	car
wide wing (ww)	ble	car
charcoal (char)	ble	car
ebony (eb)	ble	car
sex (M)	ble	eagle (eag)
sex (M)	ble	bronze (brz)
eb/fr	ble	car
mul/fr	ble	car
sex (M)	ble	melonotic-2 (mel-2)
sex (M)	ble	car/brz
eb/ww	ble	car
char	ble	car/eag
sex (M)	ble/melonotic-1 (mel-1)	car
fr/ww	ble	car
eb	ble	car/eag

Table 4. Results of laboratory matings when mutant <u>Culex</u> <u>tarsalis</u> females were caged with "wild" males in competition with either mutant or translocated males.

Cross	Mutant ♀♀	Wild ♂♂♂		Mutant ♂♂♂		Translocated mutant ♂♂	
	Genotype	Genotype	No. matings	Genotype	No. matings	Genotype	No. matings
I	<u>car</u>/<u>car</u>; <u>ble</u>/<u>ble</u> (44)	<u>+</u>/<u>+</u>; <u>+</u>/<u>+</u> (44)	10	<u>car</u>/<u>car</u>; <u>ble</u>/<u>ble</u> (44)	14		
II	" " (44)	" " (44)	14			<u>car</u> <u>ble</u> T15<u>A</u>/<u>car</u> <u>ble</u> T15<u>A</u> (44)	7
III	" " (45)	" " (45)	5	" " (45)	15		
IV	" (45) "	" (45) "	23*			<u>car</u> <u>ble</u> T16<u>A</u>/<u>car</u> <u>ble</u> T16<u>A</u> (45)	3*
V	" " (64)	<u>+</u>/<u>car</u>; <u>+</u>/<u>car</u> (64)	11	" " (64)	3		
VI	" " (64)	" " (64)	14			<u>car</u> <u>ble</u> T15<u>A</u>/<u>car</u> <u>ble</u> T16<u>A</u> (64)	6

* Includes 3 matings with both types of males; ( )= number of individuals

Table 5. Source of variation affecting the longevity of Culex tarsalis in

 1-gallon laboratory cages.

Source of variation	DF	F	Significance level
Irradiation	2	37.449	0.001 **
Strain	1	15.205	0.001 **
Sex	1	2.857	0.091
Cage	2	128.726	0.001 **

DF=degrees of freedom; F=statistical test

Table 6. Longevity of Berkeley and PWC-78 irradiated and unirradiated *Culex tarsalis* ♂♂, and unirradiated females of the same age and strain confined with the males in 1-gallon laboratory cages.

| Treatment | | | Longevity (days) | | Number of |
Strain	Dose (kr)	Sex	$\bar{x} \pm SD$	Range	mosquitoes
Berkeley	0.0	M	18.60 ± 7.42	1 – 34	60
Berkeley	0.0	F	17.77 ± 7.12	3 – 34	60
Berkeley	5.0	M	20.40 ± 11.23	3 – 41	60
Berkeley	5.0	F	23.10 ± 12.21	1 – 48	60
Berkeley	7.0	M	20.72 ± 6.92	3 – 31	60
Berkeley	7.0	F	21.30 ± 8.30	1 – 40	60
PWC-78	0.0	M	17.82 ± 5.50	2 – 29	60
PWC-78	0.0	F	17.50 ± 5.58	4 – 27	60
PWC-78	5.0	M	22.32 ± 8.52	2 – 57	60
PWC-78	5.0	F	23.67 ± 8.24	4 – 43	60
PWC-78	7.0	M	14.00 ± 4.74	2 – 24	60
PWC-78	7.0	F	12.10 ± 3.55	2 – 27	60

Table 7. Mean (± SD) number and range of female Cx. tarsalis mated or fully inseminated by an unirradiated or an irradiated male in 10 days. Number of replications for each colony and each radiation dose = 25.

Colony	Radiation dose (kR)	Number of mated females per cage		Number of fully inseminated females per cage	
		x ± SD	Range	x ± SD	Range
KL	0.0	5.320 ± 2.096	1-10	2.760 ± 1.739	0-6
KL	5.5	4.040 ± 1.060	2-6	1.680 ± 0.852	0-3
PWC-78	0.0	4.000 ± 1.803	0-7	2.400 ± 1.190	0-5
PWC-78	5.5	3.440 ± 1.583	0-7	1.880 ± 1.092	0-5

Table 8. Mean (± SD) number and range of spermathecal filling in 10 females during 10 days by a

Culex tarsalis male. Number of replications = 100.

Amount of spermathecal filling	Mean (± SD)	Number of females per cage	
		Range	95% C. I.
1 spermatheca	2.020 ± 1.101	0-5	1.802
2 spermathecae	1.670 ± 0.995	0-4	1.472
3 spermathecae	0.510 ± 0.674	0-3	0.376
Fully inseminated	2.180 ± 1.313	0-6	1.919
Total number mated	4.200 ± 1.792	0-10	3.844

Table 9. Percentage of egg rafts with no hatch and no embryonation produced by KL female _Culex tarsalis_ caged with an equal number of irradiated or unirradiated KL males. Mean hatch rate based on all rafts, and rafts excluding unembryonated rafts.

Radiation dose	Total no. females	Total no. rafts	% of total rafts with no embryonation	Mean hatch rate (± SD) Including no embryonation rafts	Excluding no embryonation rafts
5.0	50	34	17.9	6.2 ± 6.8	7.5 ± 6.8
5.0	50	24	37.5	4.6 ± 6.8	7.4 ± 7.4
6.0	50	39	38.5	0.6 ± 1.0	0.9 ± 1.9
0.0	40	21	4.8	75.4 ± 23.0	79.2 ± 22.7

Table 10. Mean (± SD) raft size, hatch rate and embryonation rate of all rafts, embryonated rafts,

inseminated rafts and uninseminated rafts from KL female Cx. tarsalis caged with an equal number of

KL males irradiated at 5.5kR.

Type of egg raft	Number of egg rafts	Raft size (eggs/raft)	Hatch rate (%)	Embryonation rate (%)
All Rafts	135	153.785 ± 40.562	1.939 ± 4.287	4.787 ± 7.838
Embryonated Rafts	97	159.856 ± 36.425	2.699 ± 4.856	6.663 ± 8.551
Rafts From Inseminated Females	116	155.026 ± 39.840	2.257 ± 4.549	5.572 ± 8.196
Rafts From Uninseminated Females	19	146.211 ± 45.134	0.000 ± 0.000	0.000 ± 0.000

Table 11. Observed hatch and mating competitiveness of irradiated male Cx. tarsalis at specific release ratios.

Release ratio (I :U :U)	Irradiation dose (kR)	# of rafts collected	Egg hatch per raft Expected 50%	Expected 50%	Observed 50%	Observed 50%	Mating competitiveness	$\bar{x}$ hatch
Release 1								
1:1:1	5.0	45	22.5	22.5	25.0	20.0	0.80	58%
2:1:1	5.0	40	13.3	26.7	13.0	27.0	1.04	37%
1:0:1	5.0	156	0.0	156.0	1.0	155.0	--	9%
Release 2								
9:1:5	5.5	244	24.4	219.6	56.0	188.0	0.37	30%
9:1:5	5.5	55	0.0	55.0	8.0	47.0	--	25%
1:0:1	5.5	54	54.0	0.0	52.0	2.0	--	86%
0:1:1	-							
Release 3								
9:1:5	6.0	39	3.9	35.1	3.0	36.0	1.33	13%
1:0:1	6.0	34	0.0	34.0	5.0	29.0	--	21%

Table 12. Percent mortalities in 3 microhabitats for 3 age groups of adult
C. tarsalis sequestered in 1 gallon screened cartons during
application of Vapona®.

Location and age group	Percent mortality			
	Treated canyon		Untreated canyon	
	Males	Females	Males	Females
Light Trap -				
1-2 day	36	40	16	16
1 week	16	20	0	0
2 week	32	24	20	4
Cattails -				
1-2 day	12	4	4	0
1 week	40	44	4	4
2 week	32	40	0	0
Tamarisk -				
1-2 day	4	4	4	4
1 week	4	8	0	4
2 week	4	0	0	0

Table 13. Characterization of egg rafts obtained from females which were trapped in all canyons at Breckenridge by embryonation, degree of hatch, and mean (±SD) hatch rate.

Date	Number of egg rafts				% of Embryonated rafts with low or medium hatch
	Unembryonated	Low hatch (0-20%)	Medium hatch (21-70%)	High hatch (71-100%)	
Prerelease -					
6-28	0	0	0	17	0.0
7-06	1	0	0	18	0.0
7-25	3	1	0	14	6.7
Postrelease -					
8-03	1	2	2	31	11.4
8-04	1	3	2	16	23.8
8-05	0	6	2	26	23.5
8-06	0	4	1	17	22.7
8-08	2	0	0	8	0.0
8-22	1	2	3	7	41.7
9-12	1	1	3	12	25.0
9-26	0	1	1	16	11.1
10-10	0	0	1	35	2.9

Table 14. Susceptibility of 19 _Culex_ _tarsalis_ strains for dieldrin.

Strain	Susceptibility*
fringe ebony	0.0022
melonotic black eye	0.0023
carmine black eye	0.0025
ebony	0.0029
Fort Collins	0.0034
OP-susceptible	0.0035
Yuma	0.0039
Knights Landing	0.0040
Manitoba	0.0046
charcoal	0.0058
melonotic-2	0.0060**
eagle	0.0062
wide	0.0068
black eye	0.0075
Berkeley	0.0075**
OP-resistant	0.0108**
fringe	0.0120
bronze	0.0125
carmine charcoal	0.0180

* Expressed as ppm dieldrin of LC_{50}

** Average value for 2 trials

Table 15. Culex tarsalis collected Poso West, September 1979 mark release.

Day	charcoal Marked ♀/♂	Unmarked ♀/♂	KL/T-78 ♀/♂	Reared ♀/♂	Trapped ♀/♂	Unmarked ♀/♂	Total ♀/♂
1	7 / 1	7 / 3	23 / 2	29 / 1	79 / 8	3270 / 545	3415/560
2	6 / 0	6 / 2	6 / 0	9 / 0	25 / 0	2635 / 422	2687/424
3	4 / 1	6 / 1	1 / 0	0 / 1	5 / 0	2540 / 616	2556/619
4	8 / 0	10 / 1	6 / 0	2 / 0	2 / 0	2892 / 709	2920/710
5	4 / 0	3 / 0	2 / 0	2 / 0	3 / 1	3283 / 876	3297/877
6	0 / 1	2 / 0	0	1 / 0	10 / 0	4714 / 1405	4727/1406
7	0	0	0 / 1	2 / 2	3 / 0	3224 / 1733	3229/1736
8	0	0	0	0	0	461 / 40	461/40
9	0	0	0	0	0	1109 / 61	1109/61
10	0	0	0	0	0	1027 / 24	1027/24
total	29 / 3	34 / 7	38/3	45/4	127/9	25,155 / 6431	25,428/6457

Table 16. Release, recapture, survival and dispersal of Culex tarsalis, Poso West, September 1979 mark-release.

Category	Release site	Color	No. released ♀ / ♂	No. recap'd ♀ / ♂	% recap'd ♀ / ♂	♀ mean daily surv.	♀ dispersal mean(m)	s.d.
Charcoal	8	aqua	237 / 320	29 / 3	12.2/0.9		134	94
"	8	unmarked	237 / 320	34 / 7	14.3/2.2		133	108
all charcoal			474 / 640	63 / 10	13.3/1.6		134	101
KL / T	8	blue	320 / 320	38 / 3	11.9/0.9		139	96
all lab			794 / 960	101 / 13	12.7/1.4	0.64	136	98
Reared	1	yellow	890 / 890	18 / 3	2.0/0.3		119	57
"	8	chart.	890 / 890	22 / 1	2.5/0.1		275	175
"	15	silver	890 / 890	5 / 0	0.6/0		616	273
all reared			2670 / 2670	45 / 4	1.7/0.1	0.62	251	212
Trapped	1	red	870 / 135	46 / 5	5.3/3.7		167	75
"	8	orange	980 / 65	65 / 3	6.6/4.6		266	173
"	15	purple	720 / 20	16 / 1	2.2/5.0		430	284
all trapped			2570 / 220	127 / 9	4.9/4.1	0.65	251	183
all wild			5240 / 2890	172 / 13	3.3/0.4	0.66		

Table 17.

Culex tarsalis collected during July, 1979, mark-release-recapture study at Poso West (females/males)

Day	7-8 day Red	6-7 day Yellow	5-6 day Chart	4-5 day Purple	3-4 day Aqua	2-3 day Gold	1-2 day Blue	1-3 day Orange	Trapped Silver	Unmarked
1	83/0	65/1	26/0	18/0	3/0	16/0	3/0	25/0	33/0	4633/472
2	8/2	9/0	4/0	2/0	1/0	0	0	2/0	4/0	3876/605
3	1/0	1/0	1/0	1/0	0	1/0	0	3/0	1/0	4729/1969
4	0/1	1/0	0/2	0	0/1	1/0	0	3/0	4/0	12342/7965
5	1/0	1/0	1/0	2/0	0	1/1	0	9/1	5/0	14726/9961
6	0	1/1	2/0	0/1	0	3/1	1/0	4/0	10/0	20208/11960
7	1/0	1/0	0	0	0	0	0	1/0	0	10423/4428
8	1/0	0	0	0/1	0	0	0	0/1	0	8659/2813
9	0	0	0	0	0	1/0	0	1/0	1/0	6006/2083
10	1/0	0	0	0	0	0	0	0	0	3425/1232
Tot	96/3	79/2	34/2	23/2	4/1	23/2	4/0	48/2	58/0	89027/43488
	4.9	5.9	2.6	5.1	5.6	2.3	0.5	1.2	6.2	% females recaptured
	0.13	0.12	0.17	0.37	0.82	0.19	0	0.052	0	% males recaptured
	281 m	242 m	204 m	231 m	243 m	297 m	264 m			mean dispersal distance (females)
	52%	60%	50%	16%	18%	12%	12%			autogeny rate

Figure 1. Gel-electrophoretic scan graphs of enzyme activity
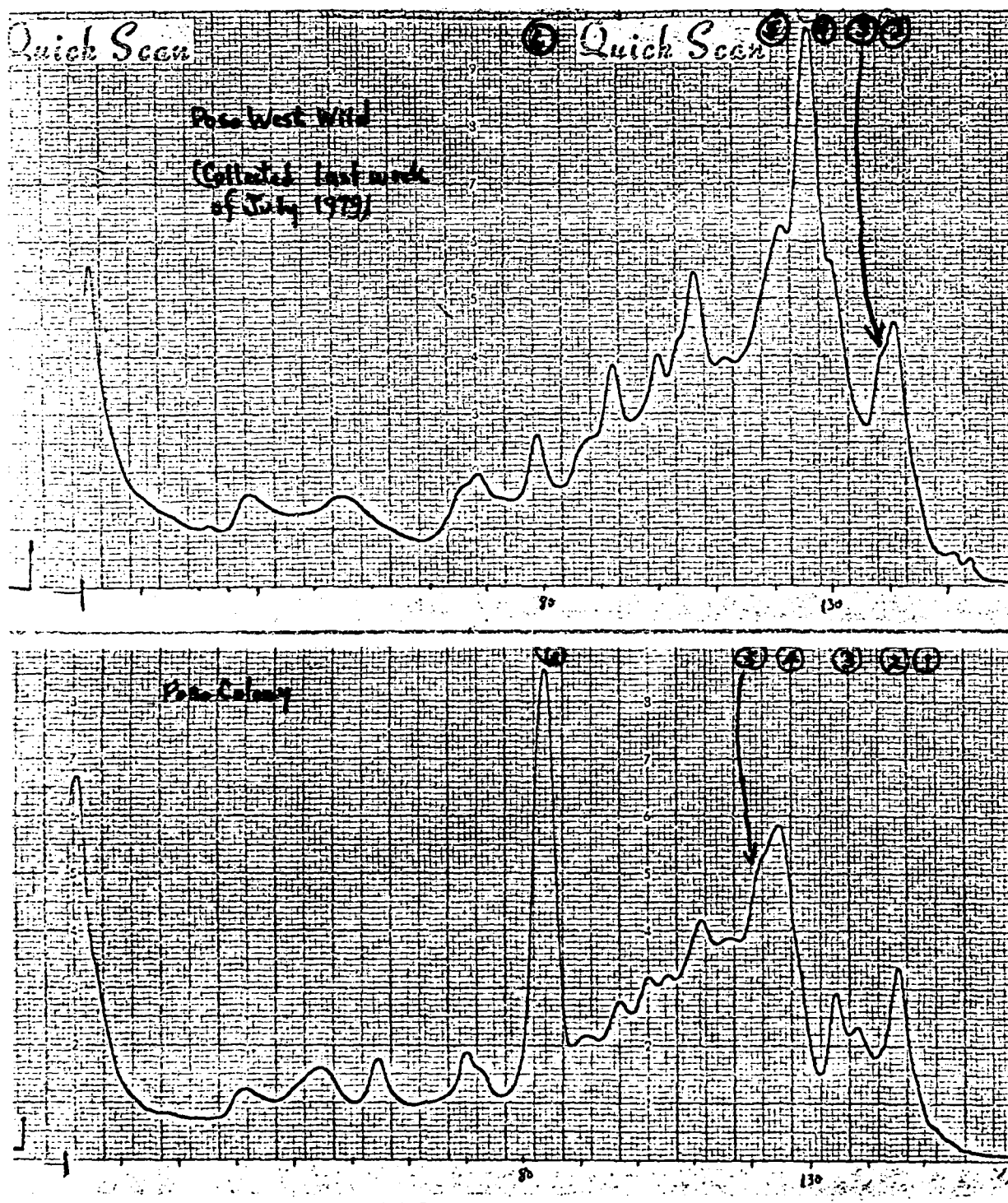in Poso West Wild and Poso West Colonized samples
of <u>Culex</u> <u>tarsalis</u>.

Figure 2. A method to capture new translocation homozygotes using existing
mutant-marked translocation homozygotes.

Step	Generation	Cross	Fertility
Irradiation	1:P	$\dfrac{car}{car}\quad\dfrac{ble}{ble}$ X $\dfrac{+}{+}\quad\dfrac{+}{+}$ (homozygote-old) (normal)	low
Heterozygote capture	2:F-1	$\dfrac{car}{car}\quad\dfrac{ble}{ble}$ X $\dfrac{car}{+}\quad\dfrac{ble}{+}$ (normal) (heterozygote-old)	medium (discard)
		$\dfrac{car}{car}\quad\dfrac{ble}{ble}$ X $\dfrac{car}{+}\quad\dfrac{ble}{+}$ (normal) (pseudohomozygote)	high (save)
Heterozygote inbreeding	3:F-2	$\dfrac{+}{car}\quad\dfrac{+}{ble}$ X $\dfrac{+}{car}\quad\dfrac{+}{ble}$ (heterozygote-new) (heterozygote-new)	low
Homozygote isolation	4:F-3	$\dfrac{+}{car}\quad\dfrac{+}{ble}$ X $\dfrac{+}{car}\quad\dfrac{+}{ble}$ (heterozygote-new) (heterozygote-new)	low (discard)
		$\dfrac{+}{car}\quad\dfrac{+}{ble}$ X $\dfrac{+}{+}\quad\dfrac{+}{+}$ (heterozygote-new) (homozygote-new)	medium (discard)
		$\dfrac{+}{+}\quad\dfrac{+}{+}$ X $\dfrac{+}{+}\quad\dfrac{+}{+}$ (homozygote-new) (homozygote-new)	high (save)

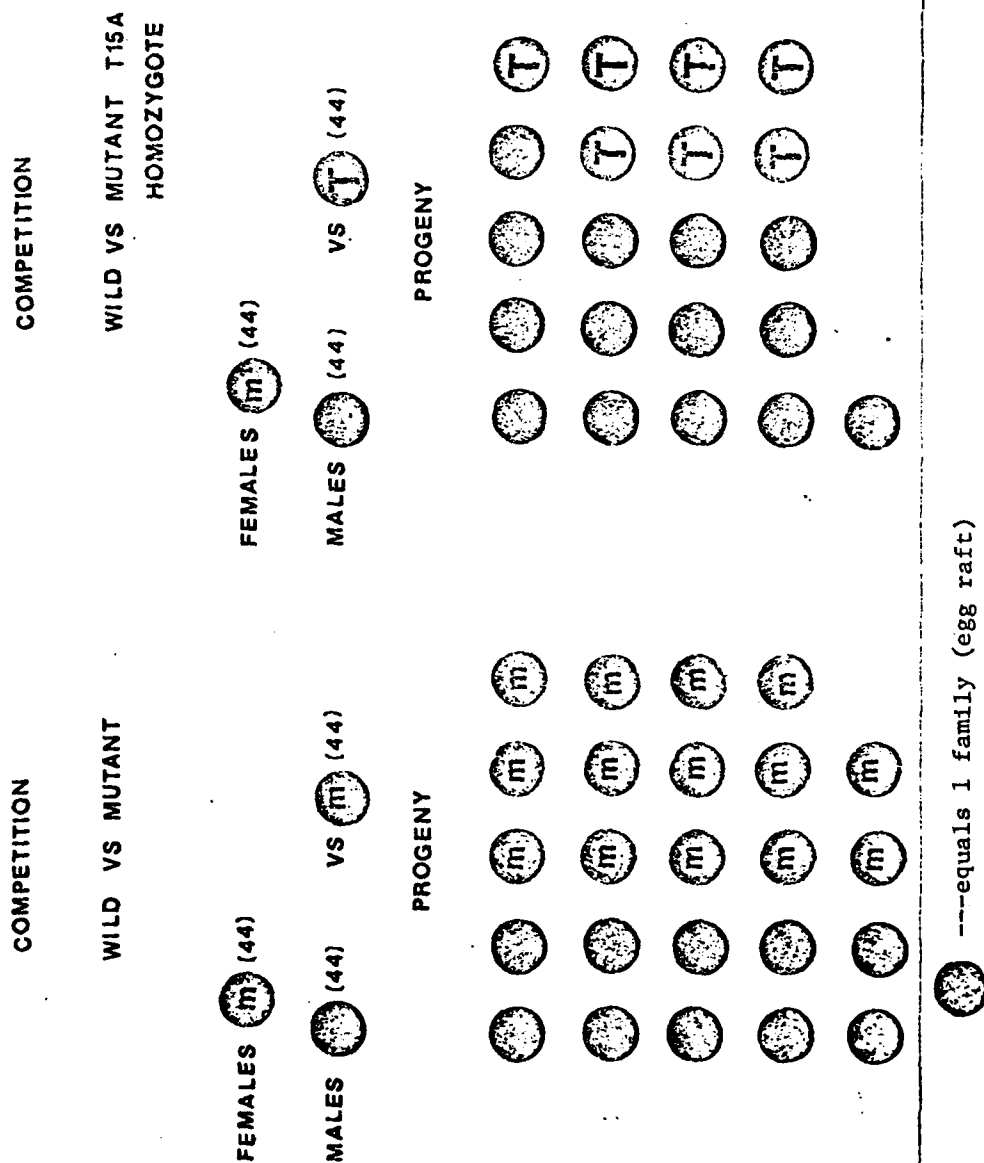Figure 3. Laboratory competition studies: wild versus mutant and mutant-homozygote T(2;3)15A males.

Figure 4. Laboratory competition studies: wild versus mutant and mutant-homozygote T(2;3)16A males.

Figure 5. Laboratory competition studies: wild versus mutant and mutant hybrid (T15A/T16A) males.

COMPETITION
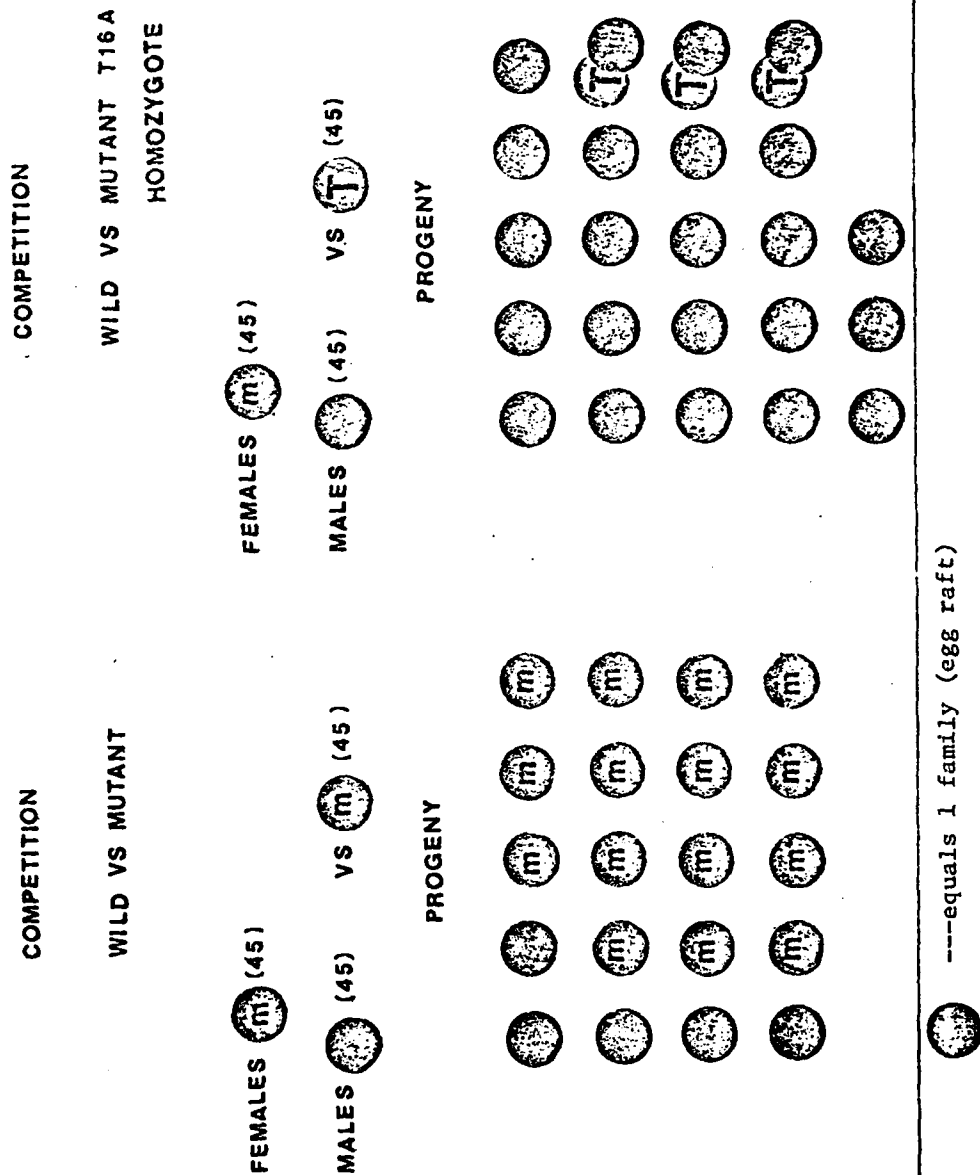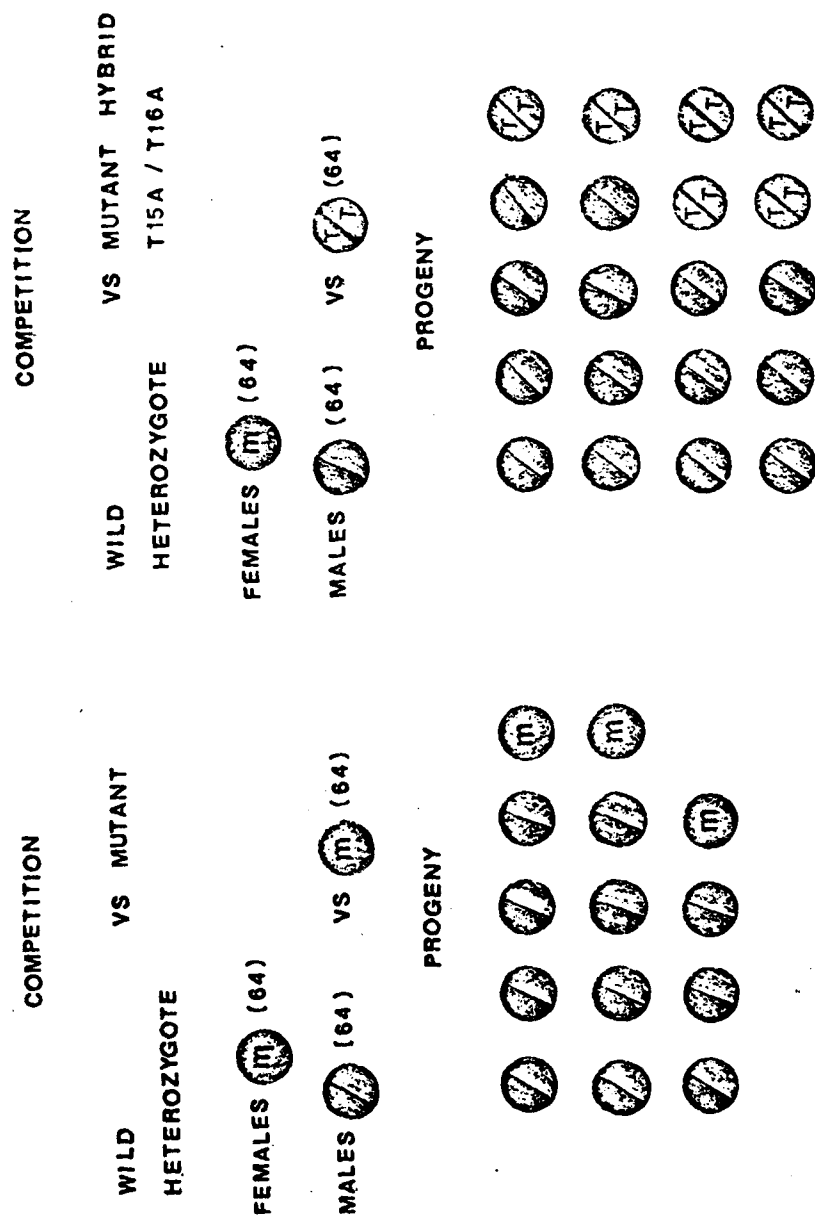
WILD   VS MUTANT
HETEROZYGOTE

FEMALES (m) (64)

MALES (//) VS (m) (64)

PROGENY

COMPETITION

WILD   VS MUTANT HYBRID
HETEROZYGOTE   T15A / T16A

FEMALES (m) (64)

MALES (//) (64) VS (T/T) (64)

PROGENY

(//) ---equals 1 family (egg raft)

Figure 6. SEQUENTIAL APPROACH TO THE SMR CONTROL METHOD

PHASE I

1. Small-scale laboratory rearing

2. Sterility curves, competition and population suppression studies

3. Improving artificial rearing techniques

4. Field-cage trials

Relevant ecological and biological studies

Large-scale wild collections

Sampling methods

PHASE II

Pilot factory for mass rearing

Quality trials in large outdoor cages

Island eradication or suppression

Release methodology

PHASE III

Factory development and release

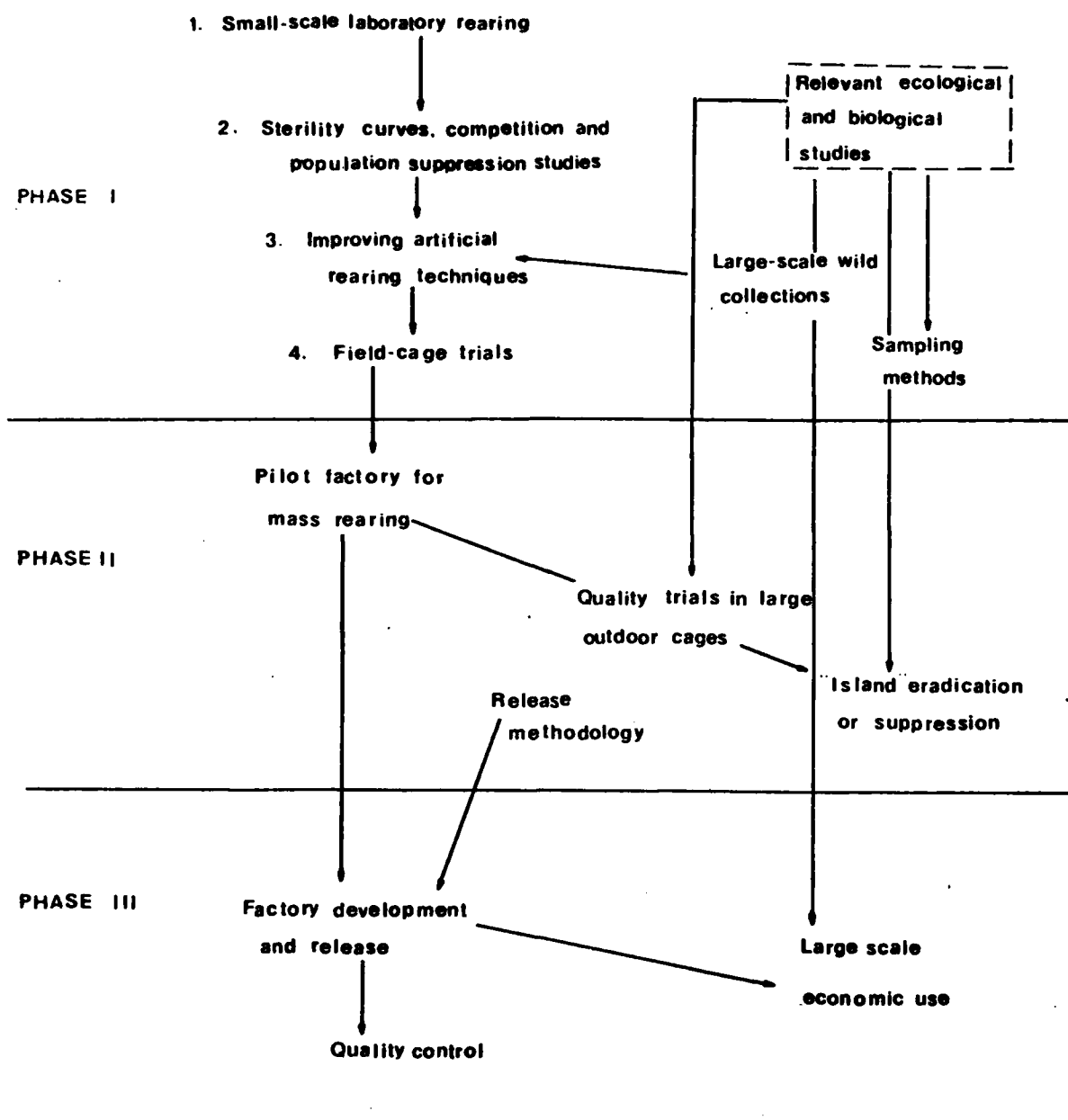Large scale economic use

Quality control

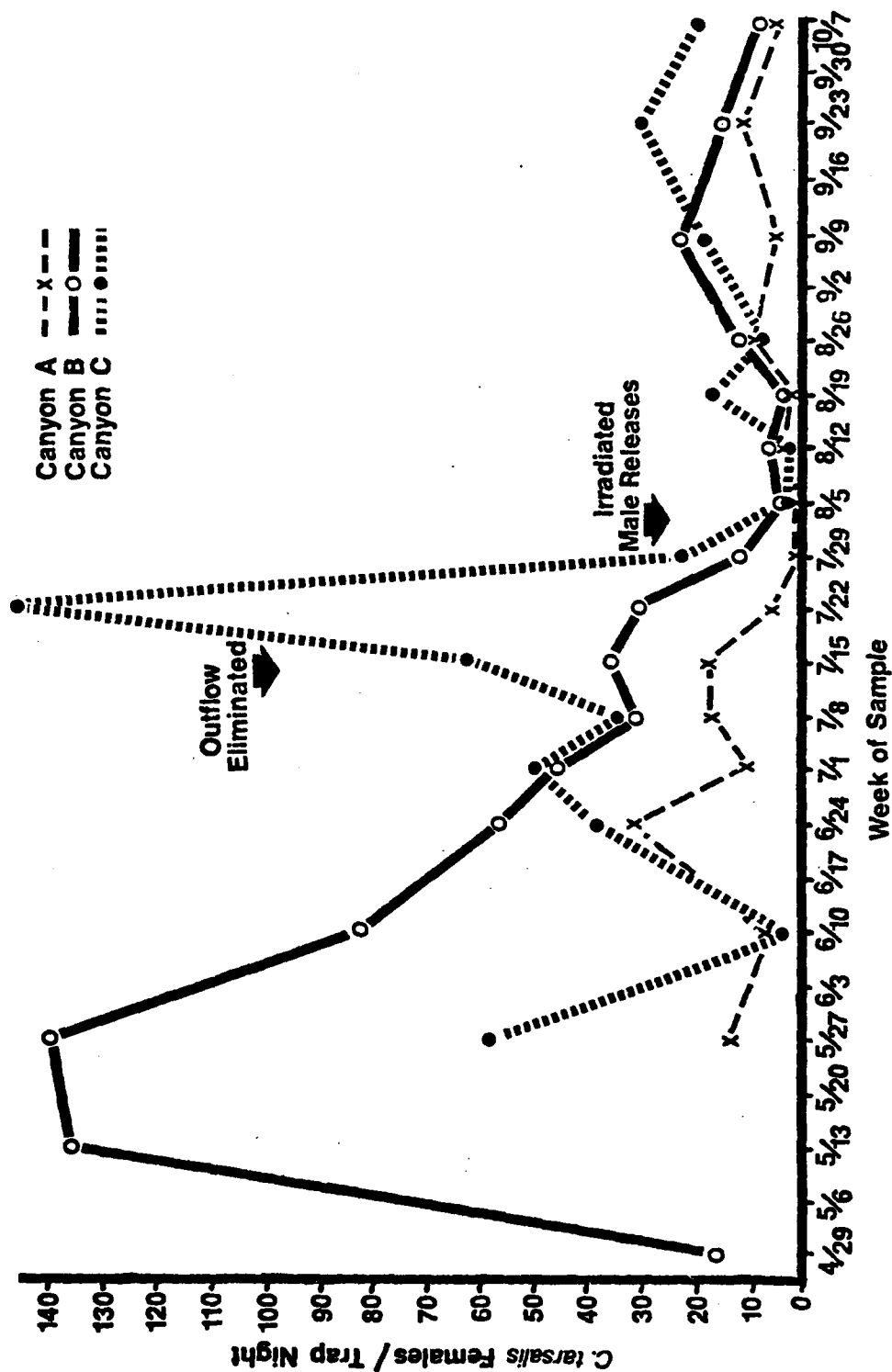Figure 7. Index of female *Culex tarsalis* at Breckenridge release site.

Figure 8. Dispersal and migration of native females at Breckenridge release site.
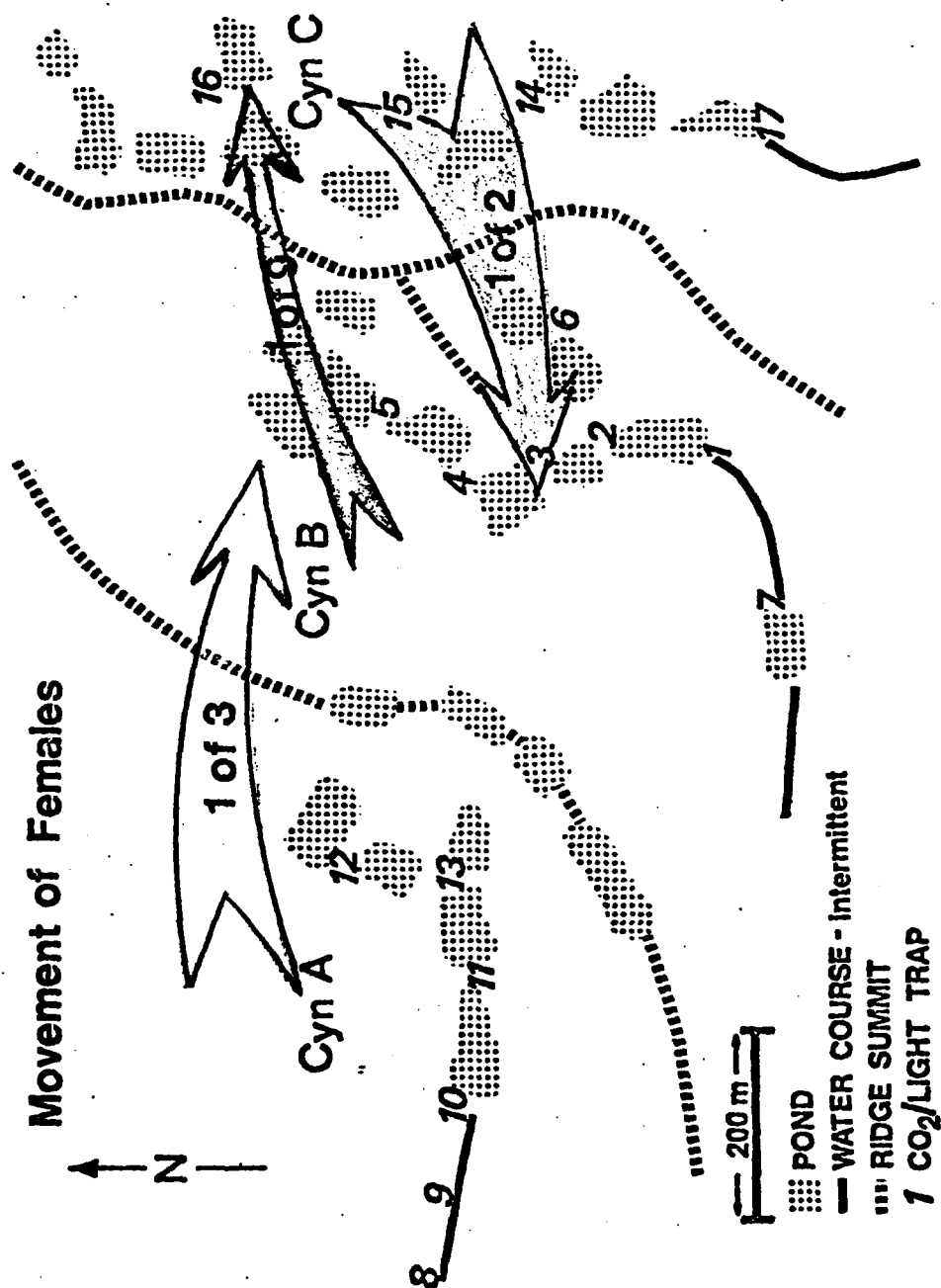
Figure 9. Points of release of sterilized males at Breckenridge release site.

Released Males:
Day 1 – 8550
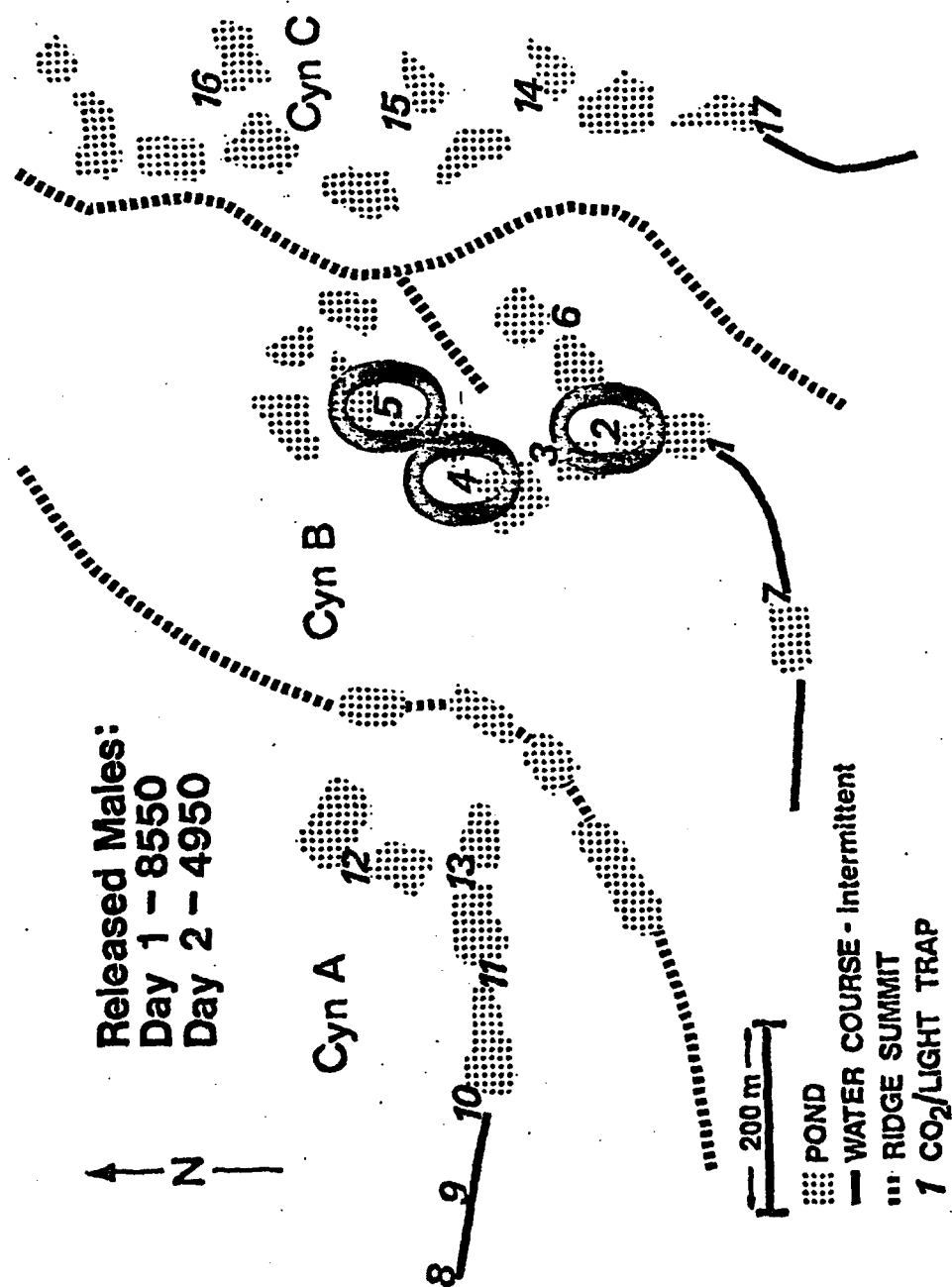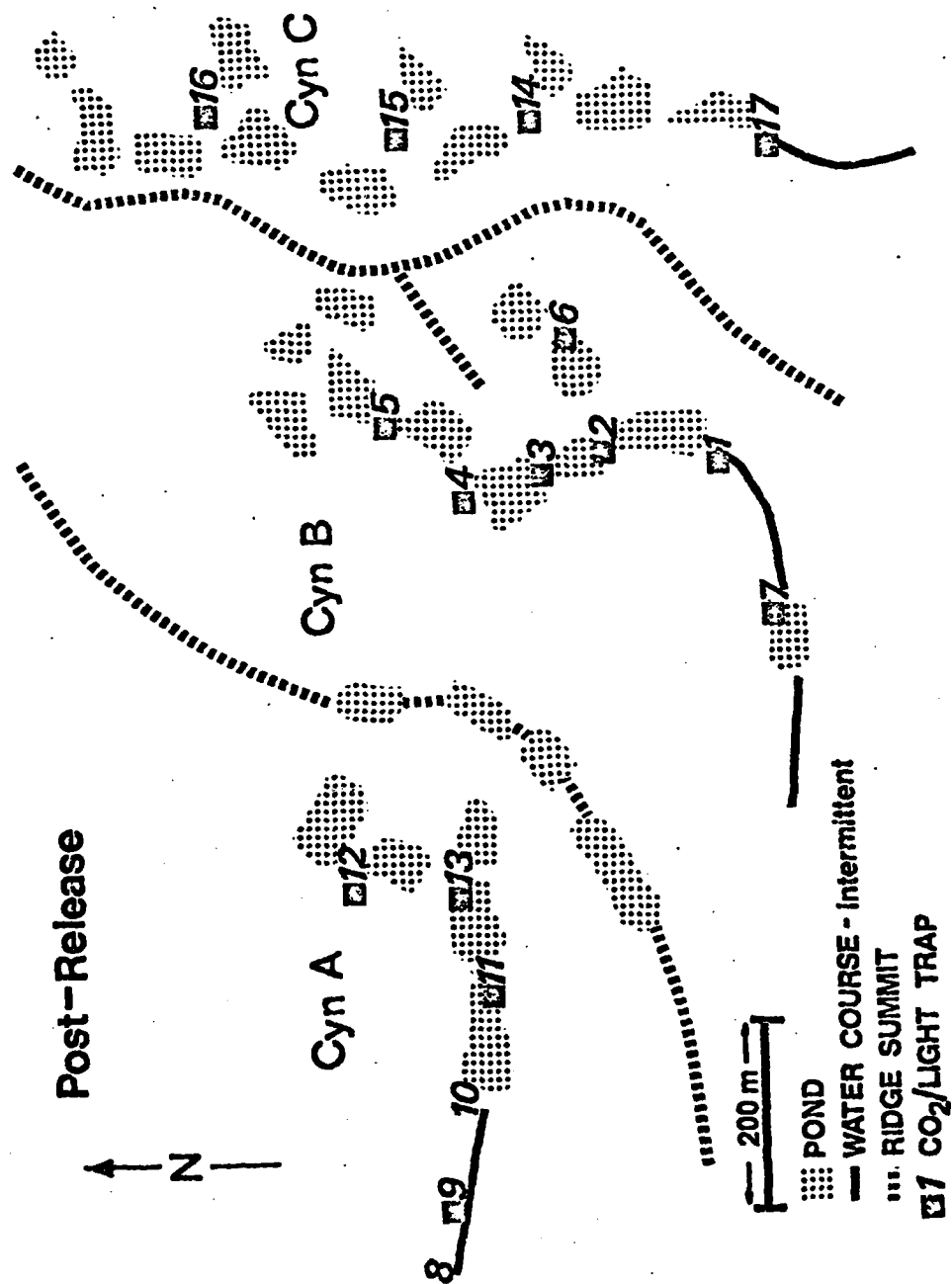Day 2 – 4950

N

Cyn A

Cyn B

Cyn C

200 m

POND
WATER COURSE - Intermittent
RIDGE SUMMIT
1 CO₂/LIGHT TRAP

Figure 10. Positions of CO_2/light traps at Breckenridge release site for recapture studies.

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